

4.4. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Determination by Broth Microdilution and Resazurin Colorimetric Assay

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 An abbreviated version of this protocol was published in *Molecules* in Feb 2021

Tannic Acid-Stabilized Silver Nanoparticles Used in Biomedical Application as an Effective Antimelioidosis and Prolonged Efflux Pump Inhibitor against Melioidosis Causative Pathogen

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Detailed protocol

Dear the reader,

Thank you so much for your interesting in our work. We are not sure that this protocol could be used with non-liquid wound dressings. We suggest the reader to use another specific protocol that could be used with a solid wound dressing, for sure, it would be more effective. By the way, it would be much appreciated to share about our detailed protocol as a request.

MICs and MBCs of all antimicrobial agents against *B. pseudomallei* (strain 1026b) and *E. coli* (O157: H7, used as a comparative reference bacteria) were performed by the broth microdilution method recommended by the Clinical and Laboratory Standards Institute [reference is cited in the paper] and described by Irazabal, et al. [Reference is cited in the paper].

For detailed protocol, bacterial cultures (overnight inoculum) were adjusted in MHB (5mL) to McFarland 0.5 turbidity standard (to reach OD of 0.001 or $1-5 \times 10^5$ CFU/mL as the bacterial starting cells) measured by uv-vis spectrophotometer or microplate reader at 630 nm. The equal volume of each antibacterial agent (50 μ L) at a final concentration of 4, 8, 16, 32, 64, 128, 256, 512 μ g/mL and adjusted bacterial solution (50 μ L) were added in each well of the 96-well plate. Ceftazidime antibiotic was used as a positive control. The plate was then incubated in an incubator at 37 °C for 18–24 h.

After overnight incubation, MIC and MBC determination in different strains of *B. pseudomallei* (1026b, H777 and 316c) with broth microdilution and resazurin colorimetric assay were proposed by Silveira, et al. and Teh, et al. [references are cited in the paper]. Broth microdilution was carried out with the same procedure as above (the same 96-well plate as above). 0.01% resazurin (7-hydroxy-3H-phenoxazine-3-one 10-oxide; Sigma-Aldrich) was added to all wells (10 μ L each well) and incubated at 37 °C for another 2–4 h until the color was completely changed. After that, the color change was observed. The result could be interpreted as the lowest concentration before the color change was determined as the MIC, the blue color represented no growth of bacteria and the pink one meant bacterial survival due to the enzymatic activity in the bacterial cells.

If you have any further questions, please don't hesitate to ask. We would be very pleased to clarify all of your questions.

Kind regards,
Oranee and co-workers

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Srichaiyapol, O. and Patramanon, R. (2022). 4.4. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Determination by Broth Microdilution and Resazurin Colorimetric Assay. *Bio-protocol Preprint*. bio-protocol.org/preprint1804.
2. Srichaiyapol, O., Thammawithan, S., Siritongsuk, P., Nasompag, S., Daduang, S., Klaynongsruang, S., Kulchat, S. and Patramanon, R. (2021). Tannic Acid-Stabilized Silver Nanoparticles Used in Biomedical Application as an Effective Antimelioidosis and Prolonged Efflux Pump Inhibitor against Melioidosis Causative Pathogen. *Molecules* 26(4). DOI: [10.3390/molecules26041004](https://doi.org/10.3390/molecules26041004)

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